

# Enthalpies of Solution of the Nucleic Acid Bases.

## 5. Adenine in Aqueous Hydrochloric Acid, Aqueous Sodium Hydroxide, Methanol, and Ethanol

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January 3, 1979

The following values were obtained from measurements of the enthalpy of solution of a well characterized sample of crystalline adenine in various solvents:

Solvent		$\Delta H(\infty, 298.15\text{K})/\text{kJ} \cdot \text{mol}^{-1}$	$\Delta C_p/\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$
HCl(aq)	1st protonation	$11.41 \pm 0.18$	$107 \pm 3^*$ (at $1 \text{ mol HCl} \cdot \text{L}^{-1}$ )
	2nd protonation	$19.3 \pm 4.4$	
NaOH(aq)	1st proton dissociation	$15.21 \pm 0.06$	$126 \pm 8^*$ (at $0.9 \text{ mol NaOH} \cdot \text{L}^{-1}$ )
	2nd proton dissociation	$17.26 \pm 0.50$	
CH <sub>3</sub> OH		$21.6 \pm 0.9^*$	$93 \pm 14$
C <sub>2</sub> H <sub>5</sub> OH		$22.0 \pm 0.9^*$	$240 \pm 40^*$

\* Uncertainty estimated (others are at the 95% confidence level).

Using these measured values the following enthalpies of protonation and proton dissociation were calculated:

	$\Delta H(\infty, 298.15\text{K})/\text{kJ} \cdot \text{mol}^{-1}$
Addition of 1 proton	$-22.1 \pm 1.1$
Addition of 2 or more protons	$-14.2 \pm 2.2$
Removal of 1 proton	$37.6 \pm 1.0$
Removal of 2 protons	$95.5 \pm 1.0$

Keywords: Adenine; enthalpy of dissociation; enthalpy of protonation; enthalpy of reaction; enthalpy of solution; nucleic acid bases; solution calorimetry; thermochemistry.

### 1. Introduction

The preceding papers in this series [1, 2]<sup>1</sup> described the results of measurements of the enthalpies of solution in water for some of the bases of the nucleic acids. In this work the enthalpies of solution or reaction of adenine (Ade), C<sub>5</sub>H<sub>5</sub>N<sub>5</sub> or 6-amino purine, in other solvents are reported.

Enthalpies of protonation and of proton dissociation of Ade have previously been obtained by various methods including titrations and spectrophotometry at various temperatures and calorimetric titrations [3]. However, the Ade samples used in the earlier work were not characterized as were the samples

used in this work. We have measured enthalpies of reaction for Ade in aqueous HCl and in aqueous NaOH. The concentration range for the HCl and NaOH was  $0.1 \text{ mol} \cdot \text{L}^{-1}$  to more than  $6 \text{ mol} \cdot \text{L}^{-1}$ . At about  $5(\text{mol HCl})\text{L}^{-1}$  there was evidence of a second protonation of Ade; this was suggested by Albert and Brown [4] as occurring at  $\text{pH} < 1$ . At about  $4(\text{mol NaOH})\text{L}^{-1}$  evidence of a second proton dissociation of Ade was observed; this has not been previously reported.

Adenine was found to be less than half as soluble in ethanol (EtOH) as in methanol (MeOH). A concentration dependence of the enthalpy of solution was observed in the range of about 1 to 8 mmol Ade (kg MeOH)<sup>-1</sup>.

Values for  $\Delta C_p$  were determined for the reactions of Ade in each of the four solvents.

<sup>1</sup> Figures in brackets indicate literature references at the end of this paper.

## 2. Materials, Apparatus, and Procedures

The adenine sample, Ade 2, previously described in detail [1] was used as received from a commercial source for all experiments reported in this work except one with Ade 5b, a sample recrystallized from aqueous solution [1]. The volatile matter for Ade 2, determined previously and presumed to be H<sub>2</sub>O, was  $(0.15 \pm 0.10)$  mass per cent or  $(1.1 \pm 0.7)$  mole per cent, and for Ade 5b,  $(0.40 \pm 0.05)$  mass per cent or  $(2.9 \pm 0.4)$  mole per cent. No other impurities were identified. These materials were assumed to be of high purity since the values for the enthalpy of solution at infinite dilution agreed with each other and were the highest of the six samples compared.

The calorimetric solutions of aqueous HCl and NaOH were taken from stock solutions prepared by dilution of the concentrated solutions with distilled water. The concentrations of the HCl stock solutions were determined by titrations using analytical reagent grade Na<sub>2</sub>CO<sub>3</sub> (dried 1 h at 548 K), with bromphenol blue as an end-point indicator; the solutions were boiled to remove CO<sub>2</sub> before adding the final portion of acid. Potassium acid phthalate, NBS Standard Reference Material No. 84 h, was used in titrations to determine the concentration of the NaOH stock solutions with phenolphthalein as the end-point indicator. CO<sub>2</sub>-free air was bubbled through the solutions during these titrations.

The platinum sample holder with interchangeable cylinders has been described [5]. In this work only the smallest cylinder, of 0.7-mL capacity, was used. It was filled in the laboratory atmosphere controlled at  $(295.6 \pm 0.5)$ K and  $(30 \pm 10)$  per cent relative humidity.

The platinum-lined adiabatic solution calorimeter, the measurement procedures, and the methods of calculation have been previously described [5, 6]. The physical constants and calibrations were the same as those given in [1]. The experiments reported here were made intermittently during the period from April 1974 through May 1976. Most of these reactions were endothermic except those in solutions above 2 (mol HCl)L<sup>-1</sup> which were exothermic. Electrical energy was not added during the endothermic reactions (except as noted in one experiment) to prevent a decrease in the calorimeter temperature, because the energy absorbed was always less than 50 J and the decrease in the calorimeter temperature was less than 0.04 K; thus, heat transfer corrections were usually negligible.

## 3. Solution of Adenine in Aqueous HCl

The enthalpy of solution of adenine was measured in 24 experiments over the concentration range of 0.1 to 6.6 (mol HCl)L<sup>-1</sup>. The results obtained in these experiments are given in table 1. The column headings are defined as follows:

Expt. No. is a serial number for experiments with this calorimeter and indicates the chronological order of the experiments.

Mass of Ade has been corrected for the H<sub>2</sub>O in the samples; all weighings were corrected to vacuum.

Mass and Concentration of aqueous HCl solutions used as the calorimetric solutions are given.

$T_{\text{reaction}}$  is the mean temperature of reaction.

Reaction period is the elapsed time between the initiation of the reaction and the beginning of the rating period which follows the reaction.

Cal. Unc., the estimated calorimetric uncertainty for an experiment, is based on the duration of the reaction, the magnitude of the temperature change from the reaction, and the standard deviation of the slope of the rating period following the reaction (see [1] for details).

Electrical energy equivalents of the initial and final systems,  $\epsilon_i$  and  $\epsilon_f$ , are given.

$\Delta T_{\text{reaction}}$  is the corrected temperature change for the reaction (see [5]).

$Q_{\text{reaction}}$  is the heat of the solution reaction represented by the following equation:

$$Q_{\text{reaction}} = \Delta T_{\text{reaction}} \left( \frac{\epsilon_i + \epsilon_f}{2} \right) - q_{\text{vap}}$$

where  $q_{\text{vap}}$  is the heat of vaporization of the solution into the air space in the sample holder upon opening and

$$q_{\text{vap}} = \Delta H_{\text{vap}} \left( V - \frac{s}{d} \right) (1 - RH)$$

where  $\Delta H_{\text{vap}}$  is the enthalpy of vaporization of the solution per unit volume at the mean temperature of reaction,  $V$  is the internal volume of the sample holder,  $s$  is the mass of sample,  $d$  is the density of the sample (for Ade,  $d = 1.47 \text{ g} \cdot \text{mL}^{-1}$  [1]), and  $RH$  is the relative humidity of the atmosphere in which the sample was transferred to the sample holder.

$\Delta H_m(T)$  is the enthalpy of solution at the temperature and concentration of the measurement, and

$$\Delta H_m(T) = -Q_{\text{reaction}} \cdot M (\text{Mass of Ade})^{-1}$$

where  $M$  is the molecular mass of adenine.

Corr. to  $T = 298.15 \text{ K}$  is  $\Delta C_p(T_{\text{reaction}} - 298.15 \text{ K})$  where  $\Delta C_p$  is the thermal coefficient determined for the reaction.

$\Delta H_m(298.15 \text{ K})$  is the enthalpy of solution at the standard temperature or  $\Delta H_m(T) + (\text{Corr. to } T = 298.15 \text{ K})$ .

The value used for  $\Delta C_p$ ,  $107 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ , was calculated from Expt. Nos. 953 and 954 in which the HCl concentration was approximately  $1 \text{ mol} \cdot \text{L}^{-1}$ . The uncertainty in the  $\Delta C_p$  value is estimated to be 2.5 per cent or 3

Table 1. Data from the measurements of the enthalpy of solution of adenine in aqueous HCl.

Expt. No.	Mass of Ade <sup>a</sup>	HCl Soln.		T <sub>reaction</sub>	Reaction period	Cal. unc.	Electrical energy equivalent		$\Delta T_{\text{reaction}}$	Q <sub>reaction</sub> <sup>b</sup>	$\Delta H_m(T)$	Corr. to T = 298.15 K	$\Delta H_m(298.15 \text{ K})$
		Mass	Conc.				Initial	Final					
	g	g	mol/L	K	min	%	J/K		K	J	J/mol	J/mol	J/mol
946	0.19909	302.474	0.101	298.078	13	0.34	1728.45	1727.70	-0.009179	-15.84	10751	- 8	10759
952	.19719	302.334	.102	298.106	48	.77	1727.87	1727.50	-.009272	-16.00	10964	- 5	10969
947	.24470	302.433	.787	298.171	18	.89	1676.33	1674.13	-.007609	-12.73	7030	3	7027
948	.22705	302.418	.787	298.173	28	.95	1677.25	1676.86	-.007355	-12.31	7326	2	7324
953	.19566	307.388	.997	298.146	38	.98	1680.58	1680.14	-.005449	- 9.14	6312	0	6312
954	.19632	307.148	.997	308.920	8	.28	1682.08	1681.79	-.006467	-10.84	7461	1148	6313
955	.20432	307.330	1.980	298.206	48	6.06	1612.30	1612.33	-.001271	- 2.03	1342	6	1336
951	.20782	307.319	2.946	298.306	48	1.63	1549.82	1550.14	.003639	5.66	-3680	17	-3697
1007	.19610	308.782	3.509	298.141	38	0.85	1522.78	1522.33	.006465	9.86	-6794	- 1	-6793
1127	.19740	324.186	3.851	298.174	33	.51	1549.86	1550.19	.008101	12.58	-8612	3	-8615
956	.19548	307.262	3.944	298.316	33	.45	1492.95	1493.51	.009012	13.48	-9318	18	-9336
1008	.19975	322.778	3.991	298.140	38	.75	1542.28	1542.08	.009064	14.00	-9471	1	-9470
1128	.21586	325.470	4.194	298.188	28	.38	1537.04	1537.11	.010526	16.20	-10141	4	-10145
1130	.18850	328.966	4.809	298.186	43	.42	1515.54	1515.37	.012540	19.02	-13620	4	-13624
SECOND PROTONATION													
1005	.19524	307.460	5.130	298.194	78	.66	1434.03	1433.89	.015479	22.22	-15379	5	-15384
1006	.18931	307.425	5.130	298.246	58	.49	1434.22	1434.02	.015144	21.74	-15518	10	-15523
1133	.20607	331.755	5.401	298.210	68	.42	1492.62	1493.90	.017281	25.82	-16931	6	-16937
1129	.19074	332.711	5.592	298.216	88	.48	1488.02	1487.90	.016967	25.27	-17902	7	-17909
1132	.20291	333.994	5.794	298.208	83	.48	1482.22	1482.33	.019887	29.50	-19646	6	-19652
1131	.20011	334.339	6.026	298.213	128	.64	1472.70	1472.78	.021211	31.26	-21109	7	-21116
949	.18931	307.403	6.030	298.236	153	.77	1393.46	1393.06	.021849	30.46	-21742	9	-21751
950	.19844	307.308	6.030	298.198	104	.53	1392.85	1392.78	.023523	32.78	-22322	5	-22327
1143	.20026	332.273	6.632	298.278	103	.81	1440.10	1440.13	.026737	38.52	-25992	14	-26006
1144	.19234	332.269	6.632	298.285	113	.72	1439.60	1439.78	.024324	35.04	-24617	15	-24632

<sup>a</sup>Corrected for H<sub>2</sub>O in sample: Ade 5b in No. 1005, (0.40 ± 0.05) mass%; Ade 2 in all others, (0.15 ± 0.10) mass%—see [1].

<sup>b</sup>q<sub>vap</sub> = -0.04 J for No. 954 and -0.02 J for all other experiments, was subtracted from the measured heat of reaction.

J·mol<sup>-1</sup>·K<sup>-1</sup> which is twice the sum of the estimated calorimetric uncertainties. At other concentrations the uncertainty may be somewhat greater.

The experiments given in table 1 are listed in order of increasing concentration of the HCl solutions. The adenine samples, 0.2 g or 0.0015 mol, are Ade 2 except in Expt. No. 1005 where Ade 5b was used. In Expt. No. 954 at 308 K, q<sub>vap</sub> was estimated to be -0.04 J and in the other experiments, -0.02 J. The data in table 1 show that the reactions are decreasingly endothermic up to about 2(mol HCl)L<sup>-1</sup> after which they are increasingly exothermic. No undissolved material was observed in the final solutions.

In figure 1 are plotted the values from table 1 for  $\Delta H_m$  (298.15 K) as a function of HCl concentration. No corrections

were made in these experiments for the variations in adenine concentration, 1.4 to 1.8 mmol·kg<sup>-1</sup> solution. The adenine concentration dependence of the enthalpy of solution in water reported previously [1] was small with a large uncertainty. Examination of the values for  $\Delta H_m$  (298.15 K) in table 1 for those experiments where the same concentration of HCl solution was used revealed no consistent dependence on Ade concentration. It is assumed that the differences are due to sample inhomogeneity and experimental uncertainty.

The two straight lines shown in figure 1 were obtained by fitting linear equations to the data for  $\Delta H_m$  (298.15 K) and HCl concentration by the method of least squares, and may be represented by the following equations: for the 14 data points below 5(mol HCl)L<sup>-1</sup>,

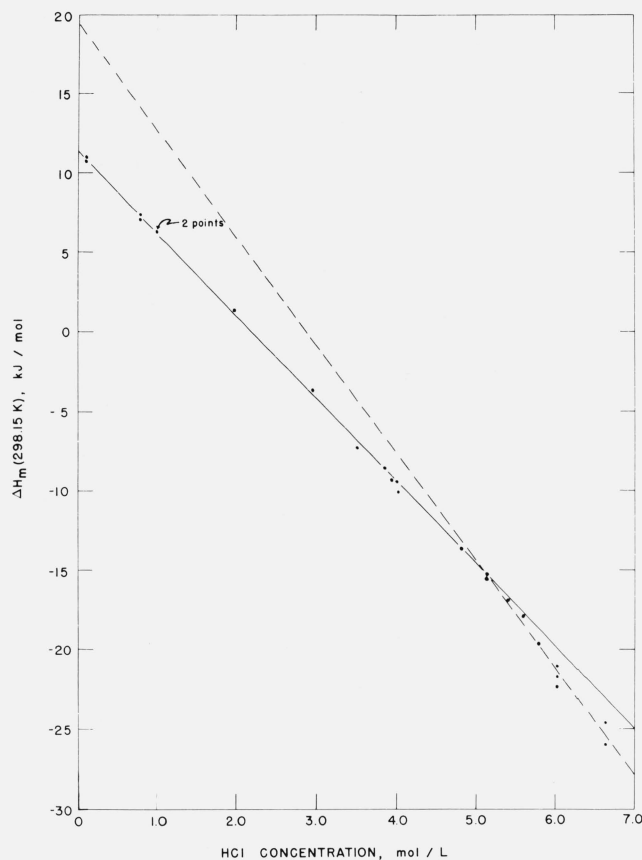


FIGURE 1. Plot showing the values for the enthalpy of solution of adenine at various concentrations of aqueous HCl solutions.

Least squares fits of linear equations to the data are represented by the solid line for the points at less than 5(mol HCl)L<sup>-1</sup>, and by the broken line for those at greater than 5(mol HCl)L<sup>-1</sup>. The change in slope is assumed to be the result of a second protonation of the adenine molecule.

$$\Delta H_m(298.15 \text{ K}), \text{ kJ} \cdot \text{mol}^{-1}$$

$$= (11.411 \pm 0.080) - (5.194 \pm 0.028)C,$$

and for the 10 data points above 5(mol HCl)L<sup>-1</sup>,

$$\Delta H_m(298.15 \text{ K}), \text{ kJ} \cdot \text{mol}^{-1}$$

$$= (19.3 \pm 2.0) - (6.76 \pm 0.34)C,$$

where  $C$  is the concentration of HCl in mol·L<sup>-1</sup> and the standard deviations of the fits are 0.17 kJ·mol<sup>-1</sup> and 0.55 kJ·mol<sup>-1</sup>, respectively. The uncertainties are the standard deviations. From the difference in the two intercepts we obtain the enthalpy of the second protonation at infinite dilution,  $(7.9 \pm 2.0) \text{ kJ} \cdot \text{mol}^{-1}$ . The change in slope at 5(mol HCl)L<sup>-1</sup> is apparently the result of the second protonation previously reported by Albert and Brown [4] at pH < 1.

It should also be noted that the length of the reaction periods given in table 1 fall into three groups:  $(30 \pm 18) \text{ min}$

for the first 14 experiments but omitting the one at 309 K,  $(73 \pm 15) \text{ min}$  for the next 5 experiments, and  $(128 \pm 25) \text{ min}$  for the last 5 experiments. This suggests a third protonation between 5.8 and 6.0 (mol HCl)L<sup>-1</sup>; however, the uncertainties in the slopes and intercepts do not justify separation of the last two groups.

#### 4. Solution of Adenine in Aqueous NaOH

In table 2 are the data for 20 experiments in which the enthalpy of solution of Ade 2 in aqueous NaOH solutions was measured. The description of column headings given in section 3 also applies to table 2. It is assumed that  $q_{\text{vap}}$  is negligibly small in these experiments because if the solution were pure water  $q_{\text{vap}}$  would be less than 0.05J in the worst case (at 309 K); the corrections are smaller than this since the vapor pressure of the NaOH solution is less than that of water.



Table 2. Data from the measurements of the enthalpy of solution of adenine in aqueous NaOH.

Expt. No.	Mass of Ade <sup>a</sup>	NaOH Soln.		T <sub>reaction</sub>	Reaction period <sup>b</sup>	Cal. unc.	Electrical energy equivalent		-ΔT <sub>reaction</sub>	-Q <sub>reaction</sub>	ΔH <sub>m</sub> (T)	Corr. to T = 298.15 K	ΔH <sub>m</sub> (298.15 K)
		Mass	Conc.				Initial	Final					
	g	g	mol/L	K	min	%	J/K		K	J	J/mol	J/mol	J/mol
960	0.19889	307.371	0.0912	298.122	23	0.38	1750.23	1749.37	0.012727	22.27	15130	- 4	15134
961	.21312	307.416	.0912	298.149	18	.31	1750.59	1750.16	.013790	24.14	15306	0	15306
1134	.19707	308.618	.197	298.193	13	.17	1744.30	1743.90	.012696	22.14	15181	5	15176
962	.19380	307.368	.462	298.127	13	.28	1725.49	1725.71	.012437	21.46	14963	- 3	14966
963	.20732	307.378	.462	298.128	18	.29	1725.26	1724.71	.013260	22.87	14906	- 3	14909
1135	.19241	308.571	.545	298.193	13	.21	1722.08	1721.82	.012416	21.38	15015	5	15010
957	.19922	307.223	.919	298.158	13	.31	1699.37	1698.97	.012974	22.04	14949	1	14948
958	.19357	307.204	.919	309.270	13	.17	1703.13	1703.74	.013703	23.34	16293	1402	14891
959	.19976	307.259	.919	298.159	18	.16	1699.38	1699.58	.012907	21.93	14834	1	14833
1139	.19726	325.979	1.998	298.105	13	.26	1725.78	1726.20	.012256	21.15	14488	- 6	14494
1138	.18930	335.734	3.012	298.102	13	.34	1734.68	1734.78	.011410	19.79	14127	- 6	14133
1148	.19732	345.010	3.582	298.101	13	.20	1756.52	1756.62	.011538	20.27	13881	- 6	13887

## SECOND DISSOCIATION

1140	.19024	346.960	4.015	298.089	13	.27	1755.71	1756.18	.011058	19.42	13794	- 8	13802
1149	.19448	350.243	4.096	298.116	13	.32	1766.38	1766.76	.011051	19.52	13563	- 4	13567
1142	.20667	348.501	4.221	298.111	18	.22	1757.53	1757.96	.011635	20.45	13371	- 5	13376
1141	.18986	353.112	4.498	298.092	12	.28	1770.04	1770.62	.010500	18.59	13231	- 7	13238
1136	.21816	313.451	5.064	298.475	8	.20	1618.02	1618.14	.012852	20.80	12883	41	12842
1137	.20246	333.433	5.064	298.113	13	.23	1691.02	1691.34	.011372	19.23	12835	- 5	12840
1145	.19739	363.371	6.078	298.095	18	.40	1786.83	1787.58	.009697	17.33	11864	- 7	11871
1147	.19118	363.306	6.078	298.081	13	.28	1787.33	1787.60	.009320	16.66	11775	- 9	11784

<sup>a</sup>Corrected for H<sub>2</sub>O in sample: (0.15 ± 0.10) mass%.<sup>b</sup>The stirring rate was 450 rpm in Expts. No. 959, 960, 962 and 963; 550 rpm in all other experiments.

The  $\Delta C_p$  for the reaction,  $126 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ , was determined from the three experiments (No. 957-959) using  $0.919 \text{ (mol NaOH)L}^{-1}$ ; an estimated uncertainty of  $8 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$  was obtained from the sum of the estimated calorimetric uncertainties (table 2) divided by the temperature difference. The uncertainty may be greater at other concentrations.

From the lengths of the reaction periods given in table 2, it can be seen that all of the reactions were rapid. No undissolved material was visible in the final solutions. The value of the enthalpy of solution decreases as the concentration of the NaOH calorimetric solutions increases. Figure 2 is a plot of  $\Delta H_m(298.15 \text{ K})$  as a function of NaOH molarity. It shows a change of slope near  $4 \text{ (mol NaOH)L}^{-1}$  which probably indicates a second proton dissociation not previously reported. The results of fitting linear equations to the data for  $\Delta H_m(298.15 \text{ K})$  and NaOH concentration from table 2 by the method of least squares are as follows:

for 12 points at less than  $4 \text{ (mol NaOH)L}^{-1}$ ,

$$\Delta H_m(298.15 \text{ K}), \text{ kJ} \cdot \text{mol}^{-1}$$

$$= (15.207 \pm 0.029) - (0.364 \pm 0.019)C$$

and for the 8 points at greater than  $4 \text{ (mol NaOH)L}^{-1}$ ,

$$\Delta H_m(298.15 \text{ K}), \text{ kJ} \cdot \text{mol}^{-1}$$

$$= (17.26 \pm 0.21) - (0.889 \pm 0.042)C$$

where  $C$  is the NaOH concentration in  $\text{mol} \cdot \text{L}^{-1}$  and the uncertainties are the standard deviations. The standard deviations of the fits are  $0.072 \text{ kJ} \cdot \text{mol}^{-1}$  and  $0.093 \text{ kJ} \cdot \text{mol}^{-1}$ , respectively. From the difference in the intercepts, we obtain the enthalpy of the second proton dissociation at infinite dilution,  $(2.06 \pm 0.21) \text{ kJ} \cdot \text{mol}^{-1}$ .

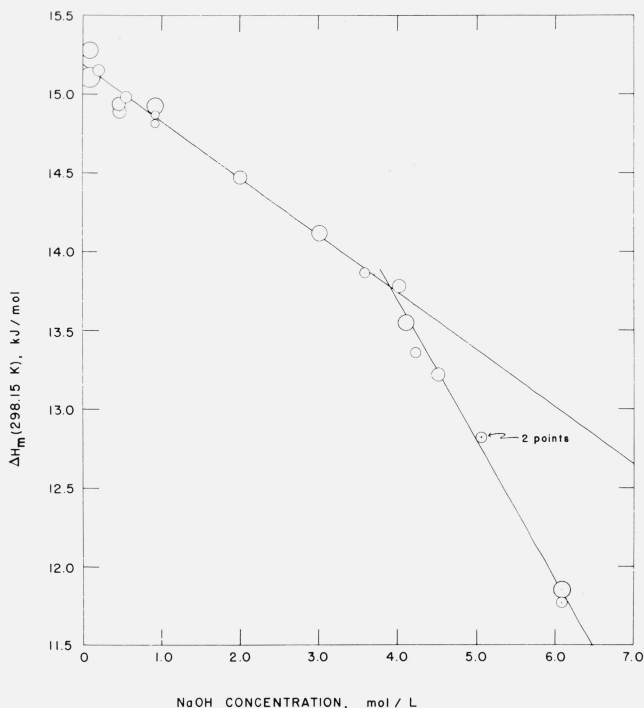


FIGURE 2. Plot showing the values for the enthalpy of solution of adenine at various concentrations of aqueous NaOH solutions.

The two lines shown were obtained by fitting linear equations to the data by the method of least squares. The change in slope at approximately  $4(\text{mol NaOH})\text{L}^{-1}$  is assumed to be the result of a second proton dissociation of the adenine molecule. The radius of a circle is equal to the estimated calorimetric uncertainty for the experiment (see text).

## 5. Solution of Adenine in MeOH and in EtOH

The data are given in table 3 for 12 measurements of the enthalpy of solution of Ade 2 in ACS Spectro-grade methanol, and for 2 measurements in ACS absolute ethanol. The column headings described in section 3 also apply to table 3.

The rating period slopes are given in table 3 because they differ markedly from those where the aqueous calorimetric solutions were used. The reaction period was preceded by the initial slope and followed by the final slope. These slopes are obtained by fitting a linear equation to about 20 time-temperature observations at 100-s intervals by the method of least squares. At 298 K, with a stirring rate of 550 rpm, the rating period slopes for the aqueous HCl solutions were between  $6.5$  and  $7.5 \mu\text{K}\cdot\text{s}^{-1}$  and for the aqueous NaOH solutions, between  $5.9$  and  $6.6 \mu\text{K}\cdot\text{s}^{-1}$ ; in both cases the final slope was  $0.1$  to  $0.3 \mu\text{K}\cdot\text{s}^{-1}$  larger than the initial slope. For the solution reaction in methanol, the final slope was always more than  $1 \mu\text{K}\cdot\text{s}^{-1}$  smaller than the initial slope even with the variations in temperature and stirring rate. This indicates an increase in the evaporation cooling effect after the reaction which is surprising in view of the fact that non-volatile molecules were added to the methanol. The solutions apparently depart greatly from ideal behavior. For

the solutions in ethanol, the initial and final slopes were essentially equal.

Some undissolved particles were visible at the bottom in all final solutions in the experiments with methanol and ethanol. The amounts appeared to be independent of both concentration and temperature of reaction. The undissolved material was not analyzed, but in one noncalorimetric experiment about 8 mg or 4 per cent of the sample mass was recovered in a filter. Most of the final solutions were cloudy, but the cloudiness decreased as the reaction temperature increased; at about 342 K, the final solutions were essentially clear. When a cloudy final solution was filtered through Whatman No. 50 hardened filter paper (for fine crystalline precipitates), the filtrate was clear indicating that the cloudiness was caused by finely divided material suspended in the solution. It is unlikely that the suspended material was adenine since its density is nearly twice that of the alcohols. The unreacted material was probably an impurity (or impurities) in the adenine which was soluble in water, but insoluble in the alcohols at the lower temperatures.

The  $\Delta C_p$  for the reaction of Ade in methanol was obtained by fitting a linear equation by the least squares method to the data for  $\Delta H_m(T)$  and  $T_{\text{reaction}}$  for 10 experiments in table 3 (omitting Nos. 1009 and 1012 because the values for  $\Delta H_m(T)$  were quite low and leakage of the sample holder was suspected). A plot of these data is shown in figure 3. The slope of the line is  $\Delta C_p = (92.9 \pm 5.3) \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ ; the uncertainty given is the standard deviation and the standard deviation of the fit is  $0.18 \text{ kJ}\cdot\text{mol}^{-1}$ .

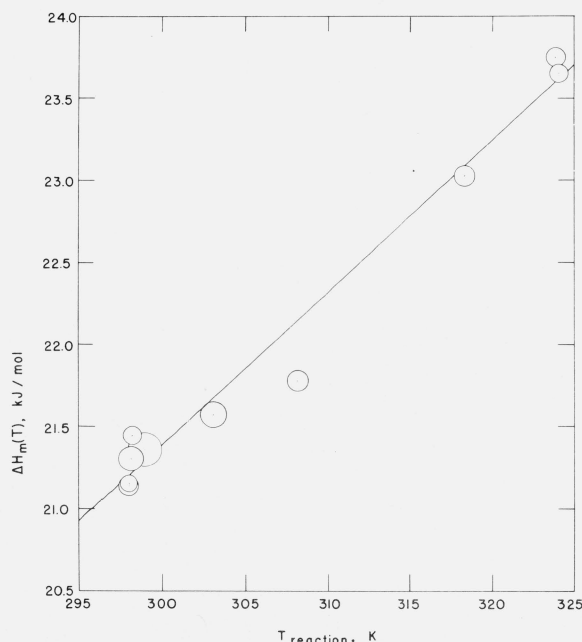


FIGURE 3. Plot showing the relationship between the enthalpy of solution of adenine in methanol and the temperature of reaction.

The straight line shown represents the result of fitting a linear equation to the data. The slope of the line,  $\Delta C_p$ , is  $(92.9 \pm 5.3) \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ . The radius of a circle is equal to the estimated calorimetric uncertainty for the experiment (see text).

Table 3. Data from measurements of the enthalpies of solution of adenine in methanol and in ethanol.

Expt. No.	Mass		Final Ade conc.	Rating period slopes <sup>b</sup>		T <sub>reaction</sub>	Reaction period	Cal. unc.	Electrical energy equivalent		-ΔT <sub>reaction</sub>	q <sub>vap</sub>	-Q <sub>reaction</sub>	ΔH <sub>m</sub> (T)	Corr. to T = 298.15 K	ΔH <sub>m</sub> (298.15 K)	Corr. to infinite diln.	ΔH <sub>∞</sub> (298.15 K)
	Ade <sup>a</sup>	Solvent		Initial	Final				Initial	Final								
	g	g	mmol/kg	μK/s		K	min	%	J/K		K	J	J	J/mol	J/mol	J/mol	J/mol	J/mol
Solvent: Spectro ACS Methanol																		
966	0.09183	242.003	2.8081	8.2	7.1	298.123	38	0.35	1086.99	1086.83	0.013508	0.18	14.50	21337	2	21339	319	21658
967	.05760	242.018	1.7613	8.0	6.7	298.973	48	1.06	1085.95	1086.59	.008568	.18	9.12	21395	- 76	21319	200	21519
969	.09479	237.012	2.9596	12.4	6.3	318.347	13	0.24	1104.19	1107.56	.015023	.44	16.18	23065	-1876	21189	336	21526
970	.09616	237.057	3.0019	10.4	6.7	308.183	23	.25	1089.34	1089.52	.014500	.28	15.52	21809	- 932	20877	341	21218
1009	.20832	237.047	6.5035	27.1	13.0	298.238	53	.23	1069.34	1071.79	.029624	.16	<sup>e</sup> 31.59	20491	36	20527	739	21266
1010	.19218	237.062	5.9993	15.0	12.6	298.013	43	.30	1072.09	1072.93	<sup>d</sup> .028238	.16	30.12	21178	- 13	21165	682	21847
1011	.20450	237.072	6.3836	14.0	12.8	298.007	38	.24	1072.03	1072.29	.030026	.16	<sup>e</sup> 32.06	21184	- 13	21171	726	21897
1012	.27306	237.047	8.5247	13.8	12.5	<sup>c</sup> 297.981	48	.27	1072.01	(1062.70)	.038458	.14	<sup>e</sup> 41.16	20368	16	20384	969	21353
1013	.14836	237.396	4.6248	13.8	12.8	298.117	38	.22	1072.31	1072.31	.022153	.17	23.58	21477	- 3	21474	526	22000
1014	.09844	237.092	3.0726	8.5	6.2	303.101	38	.34	1079.76	1079.72	.014779	.22	15.74	21606	- 460	21146	349	21495
1015	.10322	237.047	3.2224	9.4	4.7	323.820	23	.21	1114.53	1118.34	.016751	.53	18.17	23787	-2385	21402	366	21768
1016	.10153	237.047	3.1697	8.4	4.8	324.032	13	.20	1115.84	1119.12	.016422	.55	17.80	23690	-2404	21286	360	21646
Solvent: Absolute Ethanol (ACS)																		
1019	0.05061	236.771	1.5818	5.9	5.8	298.143	24	0.87	1048.20	1049.53	0.007949	0.10	8.24	22001	----	22000		
1020	.09757	236.836	3.0488	4.9	5.0	311.164	33	.60	1077.26	1078.62	.017030	.18	18.18	25178				

<sup>a</sup>Corrected for H<sub>2</sub>O in sample: (0.15 ± 0.10) mass%.<sup>b</sup>The stirring rate was 700 rpm in Expts. No. 1009 through 1013, and 550 rpm in all others.<sup>c</sup>Refers to the final temperature of the reaction since only the initial energy equivalent was used in calculating the heat of the reaction; all other experiments refer to the mean temperature of the reaction, and the mean energy equivalent was used.<sup>d</sup>Electrical energy (887.961 J) was added during this reaction; the net ΔT = 0.799691 K.<sup>e</sup>Corrections for departures of the adiabatic shield from the calorimeter temperature: No. 1009 = 0.034 J, No. 1011 = 0.027 J, and No. 1012 = 0.070 J.

Figure 4 is a plot of the adenine concentration in the final solutions versus  $\Delta H_m(298.15 \text{ K})$  before the corrections to the mass of Ade for  $\text{H}_2\text{O}$  in the sample were applied. These corrections were small and caused the line shown in figure 4 to be raised  $0.02 \text{ kJ} \cdot \text{mol}^{-1}$  with a negligible change in slope. This line was obtained by fitting a linear equation to the data from the 12 experiments by the method of least squares. For the data which includes the  $\text{H}_2\text{O}$  corrections,

$$\Delta H_m(298.15 \text{ K}), \text{ kJ} \cdot \text{mol}^{-1} \\ = (21.60 \pm 0.18) - (0.114 \pm 0.038)C$$

where  $C$  is the Ade concentration in  $\text{mmol} (\text{kg MeOH})^{-1}$  and the uncertainties are standard deviations. The standard deviation of the fit is  $0.26 \text{ kJ} \cdot \text{mol}^{-1}$ . The slope,  $113.7 \text{ J} \cdot \text{mol}^{-1} \cdot C^{-1}$  was used for the corrections to infinite dilution (table 3) which were added to  $\Delta H_m(298.15 \text{ K})$  to obtain  $\Delta H(\infty, 298.15 \text{ K}) = (21.6 \pm 0.9) \text{ kJ} \cdot \text{mol}^{-1}$ ; the large uncertainty of 4 per cent was assigned to account for the unidentified, undissolved material in the final solutions.

No corrections were made for the dilution in MeOH of the  $\text{H}_2\text{O}$  impurity in the Ade primarily because of the uncertainty in the state of the  $\text{H}_2\text{O}$ . In the worst case (Expt. No. 1012) assuming liquid  $\text{H}_2\text{O}$  in the Ade, the correction to  $\Delta H(\infty, 298.15 \text{ K})$  would be less than  $0.1 \text{ kJ} \cdot \text{mol}^{-1}$  which is much smaller than the assigned uncertainty.

In Expts. No. 1009, 1011, and 1012 (table 3), the heat of the endothermic reaction was large enough to result in small departures of the temperature of the adiabatic shield from that of the reaction vessel. Therefore small corrections for heat transfer from the shield to the vessel were made as previously described [5]. The need for this correction was eliminated in Expt. No. 1010 because precisely measured electrical energy was added during the reaction to prevent a decrease in the temperature of the reaction vessel.

In figure 4, the experiments represented by the two points at the lower right (Expts. No. 1009 and 1012) were suspected of prereaction although the rating period slopes did not support this suspicion. If these two experiments are in error, we would conclude that the enthalpy of solution was independent of concentration in the range of these measurements.

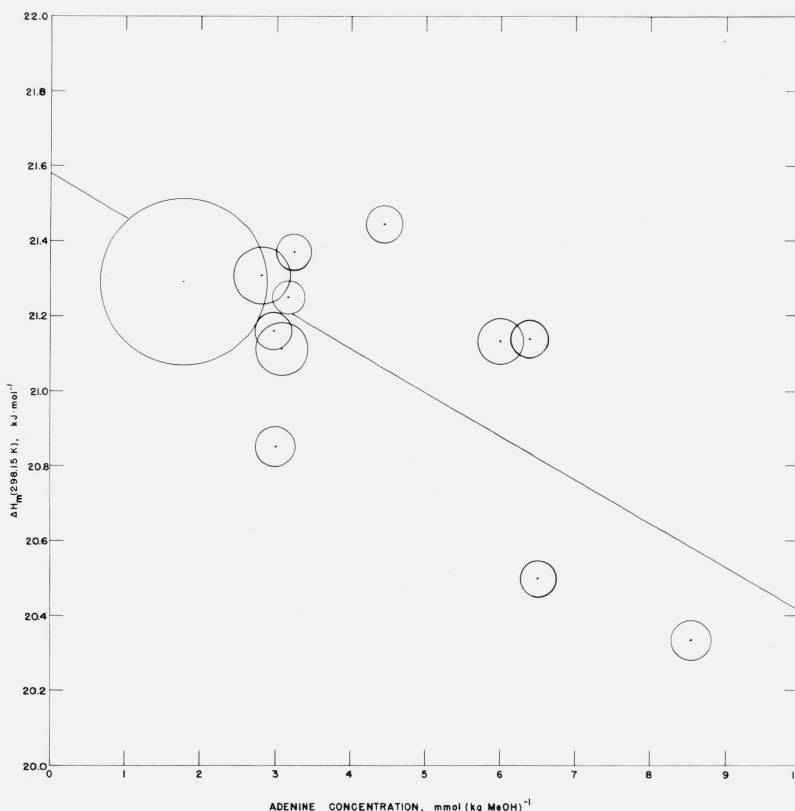


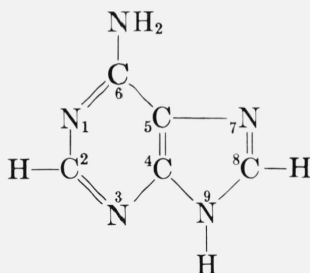
FIGURE 4. Plot showing the values for the enthalpy of solution of adenine in methanol at various concentrations of adenine.

The radius of a circle is equal to the estimated calorimetric uncertainty of the experiment (see text). The straight line shown is obtained by fitting a linear equation to the data by the least squares method; the slope is  $(114 \pm 38) \text{ J} \cdot \text{mol}^{-1} (\text{mmol Ade})^{-1} (\text{kg MeOH})$ .

In preliminary experiments, it was learned that only about one-third as much Ade could be dissolved in ethanol as in an equal volume of methanol. The final solution in Expt. No. 1020 (table 3) was very close to saturation. At such low concentrations, meaningful dilution data were not obtained. However,  $\Delta C_p$  for the reaction was about  $(0.24 \pm 0.04) \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$  and  $\Delta H(298.15 \text{ K}) = (22.0 \pm 0.9) \text{ kJ} \cdot \text{mol}^{-1}$ ; the uncertainties are estimated taking into consideration the unreacted material in the final solutions.

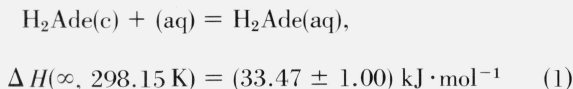
## 6. Discussion and Summary

The structure of adenine has been given as follows [4]:

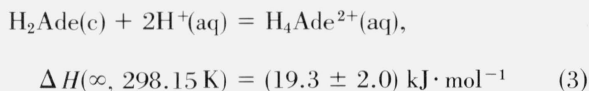
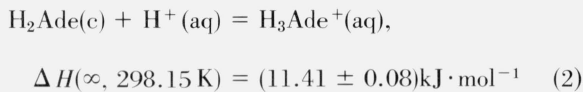


There are three possible sites for protonation at N1, N3, and N7, and two sites for proton dissociation at N9 and the amino group. A review [3] lists measurements of the enthalpy of protonation at N1 (pH 4) and suggests a second protonation [4] at N7 (pH < 1); enthalpies of proton dissociation at N9 (pH 10) are also listed.

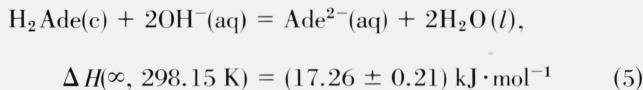
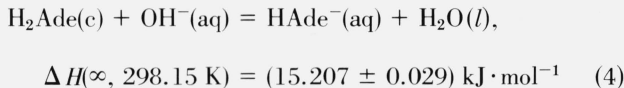
The first work in this series [1] gave our best value for the enthalpy of solution at infinite dilution which may be represented by the following equation (where adenine =  $\text{H}_2\text{Ade}$ ):



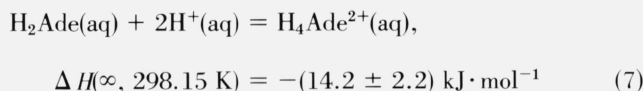
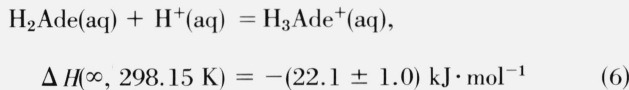
From the measurements reported in section 3 of this work we assume the following equations:



(Some evidence is mentioned in section 3 that eq (3) may represent a triple protonation rather than the addition of the two protons as indicated here.) From the measurements in section 4,



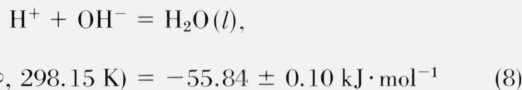
By subtracting eq (1) from eq (2) and from eq (3) we obtain



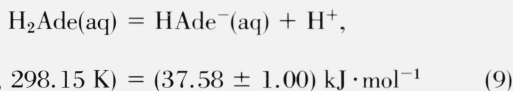
where the uncertainties are the square root of the sum of the squares of the individual uncertainties.

Equation (6) is equivalent to the protonation of N1 as given in [3]. The value obtained for this reaction by Christensen et al. using calorimetric titrations is  $-(20.12 \pm 0.08) \text{ kJ} \cdot \text{mol}^{-1}$  [7]; the purity of their sample was not determined. Equation (7) has not been previously measured although evidence for it was given by Albert and Brown [4].

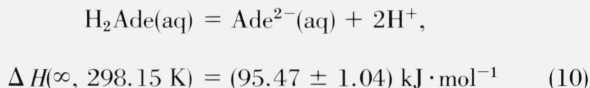
Parker [8] has selected a "best" value for  $\Delta H_N^0$  which may be represented by the equation,



If the sum of eq (1) and eq (8) is subtracted from eq (4), we obtain



which is equivalent to the value,  $(40.38 \pm 0.21) \text{ kJ} \cdot \text{mol}^{-1}$ , reported for the dissociation of the N9 proton by Christensen et al [7] using a sample of unknown purity. Similarly, if the sum of eq (1) and twice eq (8) is subtracted from eq (5) we obtain



This reaction has not been previously reported and is assumed to be the proton dissociations at the amino group and N9.

Scruggs, Achter, and Ross [9] measured the solubility of adenine in methanol at 277.8 K and 309.8 K. Assuming linearity between these temperatures, and the density of

methanol =  $0.79 \text{ g} \cdot \text{mL}^{-1}$ , we calculate  $8.0 \text{ mmol Ade (kg MeOH)}^{-1}$  for the saturated solution. (This is less than the concentration of our final solution in Expt. No. 1012 and the disagreement is probably the result of uncertainty in the purity of the adenine and of the MeOH). Scruggs et al. calculated the enthalpy of solution at  $310 \text{ K}$  for the saturated solution. Applying our value for  $\Delta C_p$  to their value for  $\Delta H$ , we obtain  $17.7 \text{ kJ} \cdot \text{mol}^{-1}$  at  $298 \text{ K}$  which is 21 percent less than our value,  $22.5 \text{ kJ} \cdot \text{mol}^{-1}$ , at  $8 \text{ mmol} \cdot \text{kg}^{-1}$ .

The following  $\Delta C_p$  values were determined for reactions of adenine:

in aqueous HCl ( $1.0 \text{ mol} \cdot \text{L}^{-1}$ ),  $(107 \pm 3) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ;  
 in aqueous NaOH ( $0.9 \text{ mol} \cdot \text{L}^{-1}$ ),  $(126 \pm 8) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ;  
 in Spectro-grade methanol,  $(92.9 \pm 5.3) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ;  
 and in absolute ethanol,  $(240 \pm 40) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ .

## 7. References

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